

The University of Chicago

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SEEDLINGS**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1935

By
LELAND BURKHART

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AMMONIUM NUTRITION AND METABOLISM OF ETIOLATED SEEDLINGS

LELAND BURKHART
(WITH ELEVEN FIGURES)

Introduction

The nitrogen metabolism of germinating seeds and young seedlings concerns itself chiefly with the decomposition of already existing proteins stored in the kernels, followed by the translocation and regeneration of the simpler substances into proteins in the meristematic and actively growing regions of the developing plant body. Studies of etiolated seedlings seem suitable for the purpose of obtaining information concerning the intermediate products of protein metabolism, since protein decomposition is favored while its regeneration is hindered in the absence of light because of the depletion of available carbohydrates.

The rôle of available carbohydrates in altering the nitrogen relations of etiolated seedlings supplied with ammonium salts has been emphasized by PRIANISCHNIKOW and his co-workers (29, 30, 31, 33, 34); however, no attempt was made to follow the changes in the carbohydrate relations quantitatively. The seedlings, grown in water cultures for a period of 10 days in the dark, were classified into three groups:

I. The cereal type (barley, maize), and the pumpkin when supplied with ammonium salts, showed increases in total nitrogen and amides, but no increase in ammonia.

II. The starchy legumes (pea, vetch) increased in total nitrogen and amides only when ammonium salts were accompanied by calcium carbonate.

III. The yellow lupine, high in protein, showed serious disturbances in the synthetic reactions, manifested by accumulation of ammonia and decrease of asparagine. Addition of calcium carbonate failed to restore the normal course of nitrogen metabolism.

PRIANISCHNIKOW deserves considerable credit for his choice of seeds with such a wide range of food reserves, which seems to have been a determinative factor in causing the different responses of the various seedlings to ammonium nutrition as reported by him. The seeds used differ greatly with respect to the relative amounts of nitrogen-free and nitrogen-containing reserves. The respective ratios used as an index to these differences are as follows: cereals, (6:1); starchy legumes, (2:1); and yellow lupine, (0.6:1).

In order to obtain additional information as to the rôle of carbohydrate reserves as affecting the response of seedlings to ammonium salts, so-called "artificial types" were employed in modifications of experiments previously mentioned. SMIRNOW (54) compared the nitrogen metabolism of etiolated

seedlings of high carbohydrate reserve (barley) and low carbohydrate reserve (yellow lupine), which were grown in sterile culture solutions. In the early stages of growth, ammonia was readily absorbed by the barley seedlings, followed by an increase in asparagine. In the later stages, when the carbohydrate reserves were exhausted (21 days), ammonia accumulated in the plants and asparagine decreased (an artificial lupine type physiologically). The presence of calcium salts promoted asparagine formation, but the processes ended sooner, as carbohydrates were more readily exhausted. The absorption of ammonium by lupine seedlings was dependent upon the presence of sugar in the medium. The addition of glucose along with the ammonium salts resulted in an "artificial physiological cereal type."

Although the results of these investigations have often been referred to in the literature, little attempt has been made to confirm these findings. The writer has previously criticized the methods employed by these workers (2). In the light of modern principles of mineral nutrition, the use of unbalanced nutrient solutions and distilled water as a control in growing their plants seems very undesirable. Calcium must play a more prominent rôle in altering the response of seedlings to ammonium salts than was realized by PRIANISCHNIKOW when he originally planned his experiments (32, 33, 38, 60). Not having separated the principal storage organs from the remainder of the seedlings, no basis was provided upon which to determine the extent of protein regeneration, all of which should be of interpretative value. The ammonia determinations are of questionable value, since the seedlings were dried prior to analysis.

With the adaptation of improved methods, this investigation involves a further study of the effects of ammonium nutrition on the carbohydrate and nitrogen relations of etiolated seedlings as altered by the type and amount of food reserves in the seed. An attempt is made to throw additional light upon certain fundamental questions: What determines the rate and amount of ammonium absorption by seedlings? How and to what extent is the ammonium utilized? What conditions are associated with so-called "ammonium injury"?

Materials and cultural methods

CHOICE AND COMPOSITION OF SEEDS

In this study it seemed desirable to employ seeds of species representing a wide range in ratios of non-nitrogenous reserves to nitrogenous reserves. Seeds were chosen in which the cotyledons served as the storage organs, thus minimizing morphological variations. Seeds of one species, with the desired range of food reserves in the various varieties, would constitute more nearly an ideal choice; however, such are not available. Variations inherent in the species of different genera might be somewhat minimized by selecting seeds

from one family, or better, one genus. The seeds used in this investigation are of the legume family except the pumpkin which was included for further study as it was found in an earlier study to respond favorably to ammonium nutrition (2). The peanut was employed with the possibility that it might compare favorably with the pumpkin on the basis of the similarity in the chemical composition of the seeds (table I), thereby having the desired range of food reserves within one family.

The following seeds were chosen:

- Pumpkin: *Cucurbita pepo* (Small Sugar variety), representing the cereal type; high oil-low protein reserves.
- Peanut: *Arachis hypogea* (Spanish variety), possible representative of the cereal type in the legume family; high oil-low protein reserves.
- White lupine: *Lupinus albus*, representing a possible intermediate between the above types and the yellow lupine; high hemicellulose—intermediate in protein reserves.
- Yellow lupine: *Lupinus luteus*: PRIANISCHNIKOW's third type; low hemicellulose-high protein reserves.

The compositions of the kernels (seed coats removed from seeds) as determined during this investigation are presented in table I. Ether extract was

TABLE I
ANALYSIS OF SEEDS

DETERMINATIONS	PUMPKIN	PEANUT	WHITE LUPINE	YELLOW LUPINE
Weight of 100 seeds (gm.)	16.65	52.08	52.47	11.52
Weight of 100 kernels (gm.)	13.16	50.90	43.80	8.72
Moisture in kernels (per cent.)	4.20	4.90	6.18	6.14
Dry weight of 100 kernels (gm.)	12.61	48.41	41.10	8.18
Percentage composition of kernels in terms of dry weight				
	%	%	%	%
Insoluble nitrogen	5.39	5.16	6.80	9.26
Soluble nitrogen	0.22	0.24	0.53	0.29
Ether extract	50.27	46.71	13.10	9.05
Starch	—	6.69	—	—
Insoluble acid hydrolyzable polysaccharides	2.88	2.84	17.04	7.76
Reducing sugars	0.13	0.08	0.08	0.12
Sucrose	3.64	2.32	3.76	1.50

determined (1) on the kernels after grinding and drying at 80° C. The methods employed in the analyses are described in a following section.

GERMINATION

The seeds were carefully selected for uniformity in size, and all those showing defects were discarded. At least 5 times as many seeds as were to be used in the experiments were germinated after preliminary treatment with 0.2 per cent. Uspulun for 20 minutes, and soaking for 2 hours in one-fifth strength nitrogen-free nutrient solution in the case of the pumpkin and white lupines. The peanuts were found to germinate better without Uspulun treatment. The yellow lupines were treated for 1 hour with concentrated sulphuric acid with subsequent washings in lime water. The germination was increased by this treatment from 15 to 90 per cent. with much more uniformity in rate of germination. After soaking for 2 hours, the seeds were placed in glass moist chambers on cellucotton kept nearly saturated with one-fifth strength nitrogen-free nutrient solution. The germination was improved by renewal of the air in the germinators at 12-hour intervals. By means of the above precautions, vigorous young seedlings free of bacteria and molds were obtained in three to four days, depending upon the species. Germination was carried out in the dark room (free of gas and laboratory fumes) in which the subsequent experiments were carried out (25° – 26° C.). The young seedlings at the above mentioned stage were selected for uniformity (radicles 3 cm. in length) and transferred to the culture solutions. Analysis of the seedlings at this stage are presented in table II. The cotyledons were analyzed apart from the remainder of the seedlings. No attempt was made to analyze the plumules separately since these made up but a small fraction. Hence the term "roots" includes the plumules with the comparatively large and fleshy radicles. From 100 to 300 seedlings were analyzed in duplicate at this stage.

NUTRIENT SOLUTIONS

LIVINGSTON (15) has stressed the need of much preliminary investigation to determine satisfactory salt combinations, concentrations, and pH before definite progress can be made in nutritional studies. In such studies complications arise; for an "optimum solution" varies among different species for a given stage of growth. Furthermore, environmental conditions alter the optimum solution for a given species and stage of growth. For example, an optimum solution for a normal seedling may be expected to differ materially from that for an etiolated seedling. It is evident that the choice of a suitable nutrient solution requires many considerations.

Just as PIRSCHLE (26, 27) has recognized the importance of the interaction of various factors on plant response to pH, one thinks of the interionic and other interacting factors as certainly affecting the response of etiolated seedlings to ammonium salts. HOAGLAND (8), LUNDEGÅRDH (16), and STEWARD (56) have briefly reviewed some of the recent papers pertaining to the various relationships involved in mineral nutrition.

TABLE II
ANALYSIS OF GERMINATED SEEDLINGS AT THE BEGINNING OF EXPERIMENTS WHEN TRANSFERRED TO NUTRIENT SOLUTIONS
(WEIGHT PER HUNDRED SEEDLINGS)

DETERMINATIONS	PUMPKIN		PEANUT		WHITE LUPINE		YELLOW LUPINE	
	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS
Fresh weight	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Soluble solids	23.2	18.0	82.5	28.2	110.0	22.2	22.1	10.0
Insoluble solids	0.71	0.19	4.50	1.81	9.30	2.24	1.42	0.46
	10.11	0.40	41.66	1.73	13.75	1.41	6.02	0.13
	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS	COTY-LEDONS	ROOTS
Insoluble nitrogen	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Soluble N	635.4	22.3	1594.0	346.4	2341.0	90.1	629.2	24.2
Amino N	42.9	13.6	337.5	246.0	326.0	80.0	101.5	33.8
Amide N	24.3	9.0	108.8	96.4	114.0	43.0	55.2	18.1
Ammonium N	7.2	3.4	32.0	24.0	32.0	35.0	27.0	10.4
Reducing sugars	1.4	0.9	5.0	3.0	9.0	3.0	1.8	0.5
Sucrose	19	29	60	351	73	129	5	32
Starch	19	6	824	480	288	129	110	15
Insoluble acid hydrolyzable polysaccharides	—	—	6333	961	—	—	—	—
	152	19	1150	22	5089	174	483	59

The question of pH of nutrient solutions requires serious consideration in connection with ammonium nutrition. The importance of the hydrogen-ion concentration as a determining factor in the absorption of ammonium and consequent response of plants has been stressed by PRIANISCHNIKOW, PIRSCHLE, MEVIUS and ENGEL, NAFTEL, and others (17, 18, 21, 26, 27, 35, 36, 37). In general, it has been shown that plants, under normal conditions, utilize ammonium most efficiently from a neutral or slightly alkaline medium; but the alkaline conditions are unsatisfactory because complications arise (precipitation, etc.). TIEDJENS (58) has indicated that pH values of 6.0–6.5 for ammonium nutrition with constantly flowing cultures were necessary for most satisfactory assimilation in the plant.

From preliminary trials the following salt combination on a partial volume-molecular basis proved suitable:

$(\text{NH}_4)_2\text{SO}_4$	0.006 M
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.004 M
KH_2PO_4	0.004 M
CaCl_2	0.004 M

A pH of 6.2 was obtained by the addition of N/10 NaOH. Proper aliquots of molar single salt stock solutions and the alkali were added to distilled water and diluted to volume just before using. The distilled water was allowed to come to dark room temperature before using. Traces of iron and boron were added. The control nutrient solution was made up in the same manner except that the ammonium sulphate was omitted.

CONTINUAL FLOWING CULTURES AND AERATION

As culture vessels 4-gallon glazed earthenware jars were used. Over each jar was placed a zinc galvanized wire net ($\frac{1}{4}$ -inch mesh) well coated with paraffin which served as an efficient support for the seedlings. A modification of the STAHL and SHIVE system of continuous renewal of nutrients (55) was adapted for use with these culture vessels. Five-gallon glass bottles calibrated at 15 liters were employed as supply and reception vessels. The rate of flow of nutrient solutions was so regulated as to allow the passage of 10 liters daily through each culture vessel, thus maintaining a constant pH. The drip solution was reused once after readjusting the pH to 6.2. The cultures were continually aerated with ammonia-free air (compressed air passed through a dilute sulphuric acid trap). The rate of aeration was such that the solution was kept in slow motion. This aeration treatment resulted in noticeably better root development. At the beginning of each experiment 250 young seedlings, selected for uniformity as previously described, were transferred to each of the 6 culture vessels (3 ammonium and 3 controls).

Methods of chemical analysis

FRACTIONATION OF PLANT ORGANS

The seedlings at each sampling were separated into stems, cotyledons, and roots. In order to make more efficient use of the plant material, the lower one-third of the hypocotyl was included with the roots in all samplings. Fifty to one hundred seedlings were sampled at each date of sampling. After obtaining fresh weights, without delay the plant material was minced into small sections (2–3 mm.) and transferred to Erlenmeyer flasks. Sufficient hot 95 per cent. alcohol was added to bring the final concentration of alcohol to 60 per cent. The contents were heated on the steam bath to gentle boiling for 20 minutes, and allowed to cool to room temperature before filtering.

EXTRACTION

Considerable difficulty was experienced in obtaining complete extraction of the soluble nitrogen with 80 per cent. alcohol, especially in the case of the white lupines (containing large amounts of asparagine) from which, even after 10 extractions, the asparagine was not completely removed. This difficulty was overcome by reducing the alcohol concentration to 60 per cent. A lower concentration resulted in filtration difficulties, especially in cotyledon material of lupines. It has previously been pointed out by DENNY (6) that unless the plant material is distinctly acidic there is little justification for employing CaCO_3 to prevent the possible hydrolysis of sucrose. In some preliminary studies it was found that young seedlings containing large amounts of sucrose, when extracted with and without the aid of CaCO_3 , gave comparable results. Accordingly, it seemed best not to include CaCO_3 ; for its inclusion results in certain complications of analysis and may possibly render extracts sufficiently alkaline to result in a loss of ammonia (if present in appreciable quantities) during the removal of alcohol to obtain water extracts for nitrogen fractions. Eight to nine extractions at 6-hour intervals secured complete extraction in 2 to 3 days. After the first extraction the residue in the flasks were, in the subsequent extractions, covered with hot 60 per cent. alcohol, heated to gentle boiling for 5 minutes and allowed to cool before filtering. After the extractions were completed and the extracts diluted to a convenient volume, nitrogen fractions were determined without delay. Thus any changes which seem to occur in nitrogen fractions during storage of alcoholic extracts (57, 63, 64) were reduced to a minimum.

ANALYSIS OF EXTRACTS

Solids and soluble nitrogen were determined on suitable aliquots.

SOLUBLE NITROGEN FRACTIONS.—The alcohol was removed from suitable aliquots, the water suspension cleared, and made to volume (2).

Ammonium nitrogen was determined on 20-cc. aliquots of the water

TABLE III
CONCENTRATION OF AMMONIUM NITROGEN IN STEMS AND ROOTS OF AMMONIUM-NOURISHED SEEDLINGS ON FRESH WEIGHT BASIS

PERIOD OF GROWTH	PUMPKIN		PEANUT		WHITE LUPINE		YELLOW LUPINE	
	STEMS	ROOTS	STEMS	ROOTS	STEMS	ROOTS	STEMS	ROOTS
3 days	%	%	%	%	%	%	%	%
6 "	0.012	0.022	0.017	0.023	0.010	0.009	0.007	0.009
9 "	0.025	0.027	—	—	—	—	—	—
12 "	—	—	0.019	0.025	0.021	0.036	—	—
14 "	0.044	0.045	—	—	—	—	0.045	0.045
16 "	—	—	—	—	0.046	0.087	—	—
18 "	—	—	0.027	0.030	—	—	—	—
30 "	—	—	0.037	0.032	—	—	—	—

extract in the Van Slyke-Cullen aeration apparatus. The aliquot was made 0.6 per cent. sodium hydroxide by the addition of 10 per cent. sodium hydroxide and aerated into 25 cc. of N/50 standard sulphuric acid, employing capryl alcohol to prevent foaming.

The ammonium-free aliquot was neutralized with glacial acetic acid added dropwise and an additional drop added to slightly acidify the mixture, which was then carefully transferred to a 50-cc. volumetric flask, the tube being carefully rinsed several times into the flask with small portions of distilled water. The flask was made to volume and the amino nitrogen determined on 2.5-cc. aliquots in the Van Slyke amino-nitrogen apparatus.

Ten-cc. aliquots of the cleared water extract were hydrolyzed (25), cooled, nearly neutralized with 1.5 cc. of 50 per cent. NaOH and allowed to recool.

TABLE IV
CONCENTRATION OF TOTAL SUGARS IN STEMS AND ROOTS OF AMMONIUM-NOURISHED SEEDLINGS ON FRESH WEIGHT BASIS

PERIOD OF GROWTH	PUMPKIN		PEANUT		WHITE LUPINE		YELLOW LUPINE	
	STEMS	ROOTS	STEMS	ROOTS	STEMS	ROOTS	STEMS	ROOTS
3 days	%	%	%	%	%	%	%	%
6 "	0.276	0.114	1.383	0.639	0.625	0.510	0.214	0.050
9 "	0.062	0.042	—	—	—	—	0.006	0.009
12 "	—	—	0.877	0.269	0.136	0.040	None	0.003
14 "	0.006	0.012	—	—	0.009	0.017	—	—
16 "	—	—	—	—	—	—	—	—
18 "	—	—	0.424	0.220	—	—	—	—
30 "	—	—	0.040	0.010	—	—	—	—

The solution was then made neutral to neutral red, and without delay made 0.62 per cent. NaOH and aerated into 25 cc. of N/50 standard acid. This gave ammonium equivalent to ammonium plus amide nitrogen. In the light of the recent findings of PUCHER *et al.* (28), VICKERY (61, 62), STUART (57), SCHLENKER (46), DAVIDSON and SHIVE (5), HULME (11), RICHARDSON (42), TOTTINGHAM *et al.* (59), the present methods of analysis of N-fractions are seriously in need of further critical examination.

SOLUBLE CARBOHYDRATES.—Direct reducing sugars and sucrose were determined on cleared and dealed aliquots of the alcoholic extracts (2). A modification of the TOMPSETT method (23) was employed in all carbohydrate determinations.

STARCH, INSOLUBLE ACID-HYDROLYZABLE POLYSACCHARIDES, AND NITROGEN.—Starch, insoluble acid-hydrolyzable polysaccharides, and nitrogen were determined on the residues (2). The peanut was the only species studied which contained starch. Dextrins were determined in the extracts of the peanut and included with the starch. Owing to the large sugar blank on the high grade commercial taka-diastase distributed by the Parke, Davis Co., it was found necessary to dialyze the preparation free of sugars before use (7). In the case of the peanut cotyledons, the residues were extracted with ether before determining starch.

EXPRESSING RESULTS.—It seemed that the results obtained in this type of investigation would in general be expressed most significantly on the basis of weight of the respective constituents per hundred seedlings. In order more conveniently to study the various trends on a comparable basis, the data were plotted graphically, thus facilitating a picturization of the relations in figures 1 to 8. The increases or decreases in the various constituents for the particular organs of a given species, resulting from ammonium nutrition, are readily shown in these figures. Certain conditions existing in the various species are more comparable when the constituents are expressed as percentage of fresh weight (tables III and IV). In order to make a comparable study of the seedlings with respect to growth response, ammonium absorption, and ammonium utilization in relation to the food reserves of the seed, certain constituents were expressed on a comparable basis of amount of constituent per 100 gm. of dry ungerminated kernels in figures 9 to 11.

SIGNIFICANCE OF RESULTS.—Having carefully selected the seeds and again the young seedlings for uniformity, variations in duplicate samples of the experimental plant material owing to individual variations of the plants were reduced to a minimum (often within the limits of error of the methods of chemical analysis (1 to 2 per cent.)), by the choice of a large number of individuals (50 to 100) per sample. Differences greater than 4 to 8 per cent. between the averages of four determinations per constituent of the two series respectively were considered significant.

Experimentation

PLAN OF EXPERIMENTS

Preliminary experiments were performed to determine the age of the seedlings of the various species at the time when starvation symptoms were apparent as the result of ammonium nutrition. Having thus determined the starvation periods for the various species, intervening sampling periods were chosen at suitable intervals in an attempt to obtain information useful in interpreting the conditions associated with ammonium injury.

Note that when the term "ammonium injury" is used in the following discussions it is not meant to imply that ammonium as such is injurious, as some writers seem to believe, but rather to convey in a convenient manner the complex of conditions associated with plants injured by ammonium nutrition. In short, the writer prefers to consider ammonium as only *one* of the various factors bringing about such pathological conditions. To be more specific, in the cases of the ammonium injury reported in the following discussions

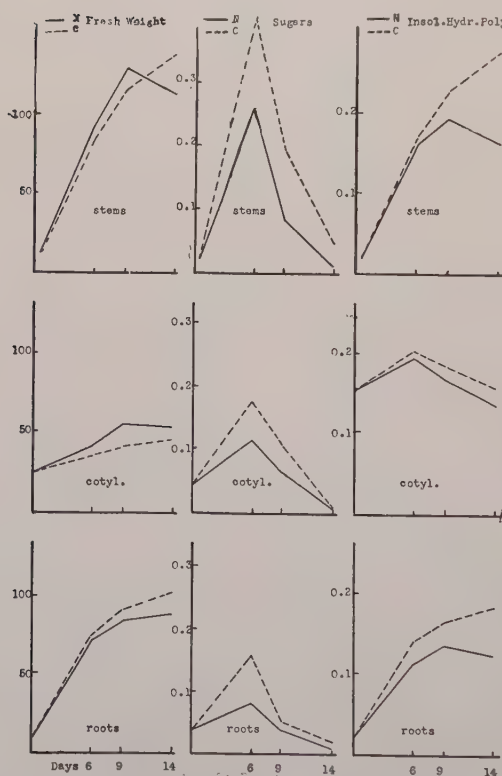


FIG. 1. Pumpkin. Fresh weights and carbohydrates in grams per hundred seedlings.

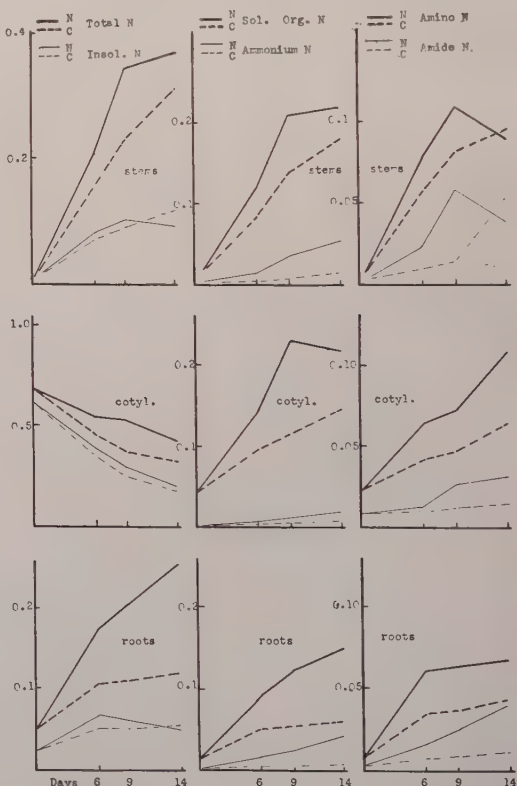


FIG. 2. Pumpkin. Nitrogen fractions in grams per hundred seedlings.

the writer prefers to consider that this condition is "injury due to extreme carbohydrate deficiency," since this would accurately express the truth; for, other conditions being favorable, including the presence of available carbohydrates, ammonium is *not toxic*.

EXPERIMENTS WITH PUMPKIN

The pumpkin seedlings were sampled at 6-, 9-, and 14-day periods. (The number of the days indicates the time elapsed from the date on which the young seedlings were transferred to the culture solutions.) The data obtained are graphically presented in figures 1 and 2.

A consideration of the fresh weight curves reveals the beneficial effects of ammonium on the growth of the stems and cotyledons during the earlier stages of growth. This favorable effect was previously noted (2). However, growth of roots was retarded. Similar growth relations were noted by REID (41) when etiolated seedlings were supplied with nitrates. The pumpkin differs materially from the other seedlings in that the cotyledons become very much expanded (growth) and succulent, and serve as very efficient photosynthetic organs in the light. At 14 days, the small leaves of the ammonium-fed epicotyls showed typical carbohydrate starvation symptoms (desiccation of the small young leaves).

The sugars were rapidly depleted in all organs by ammonium nutrition (fig. 1) with simultaneous decreases in amounts of hemicellulose. Sucrose, although present in appreciable amounts at the beginning of the experiments (table II), especially in the cotyledons as in the other species, disappeared at a much more rapid rate than the reducing sugars. Starch was absent from all tissues.

NITROGEN FRACTIONS.—The curves (fig. 2) show the remarkably rapid rate of ammonium absorption and utilization. In the early stage, protein formation especially in the roots was favored by ammonium nutrition, but not at the expense of the protein reserves of the cotyledons. It is of interest to note that the root development was retarded, even though protein formation was favored. These relations are to be expected since it has been repeatedly demonstrated by various investigators that root development is favored by higher carbohydrate conditions. In the intermediate stage (9-day) protein formation was retarded in the roots with a simultaneous increase in the stems, although not appreciable. Ammonium nutrition resulted in a sparing action on the reserve proteins at this stage. Amides strikingly had accumulated at this period while ammonium also accumulated in appreciable quantities in all organs. At the period of carbohydrate starvation (14 days) the synthetic processes were seriously disrupted as evidenced by the decrease in amino acids and amides. However, these conditions were just the reverse in the roots. The accumulation of amino nitrogen with a simultaneous

decrease in total soluble organic nitrogen in the ammonium-nourished cotyledons suggests that the amino acids accumulate at the expense of the more complex soluble compounds (polypeptides, etc.).

EXPERIMENTS WITH PEANUT

The seedlings were sampled at 6, 12, 18, and finally at 30 days when ammonium injury was apparent (starvation symptoms—desiccation of the older leaflets). Control seedlings were grown for 40 days when starvation symptoms were evident similar to those observed in the ammonium-nourished seedlings. There was no nodule formation in either series of the peanuts and lupines, and no evidence of nitrogen fixation was obtained. As is shown in figure 3, the seedlings did not respond significantly to ammonium nutrition during the early stages. The differences in amount and concentrations of sugars between the two series were amplified as the seedlings approached starvation especially in the roots. Somewhat similar trends were also appar-

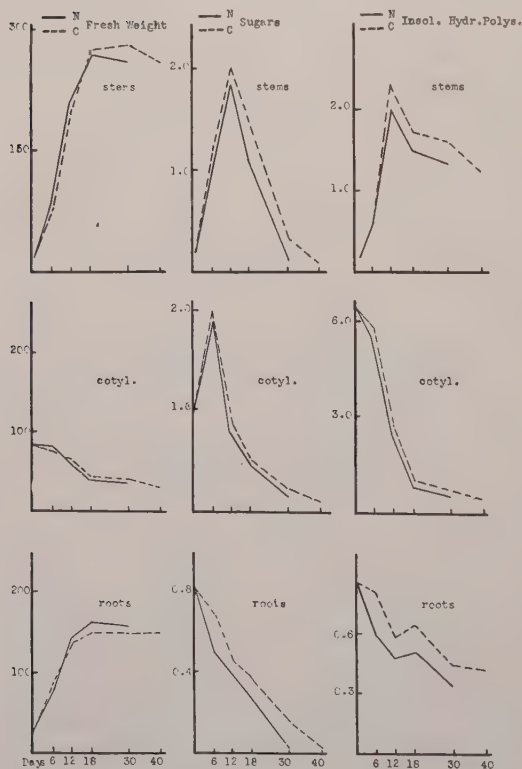


FIG. 3. Peanut. Fresh weights and carbohydrates in grams per hundred seedlings.

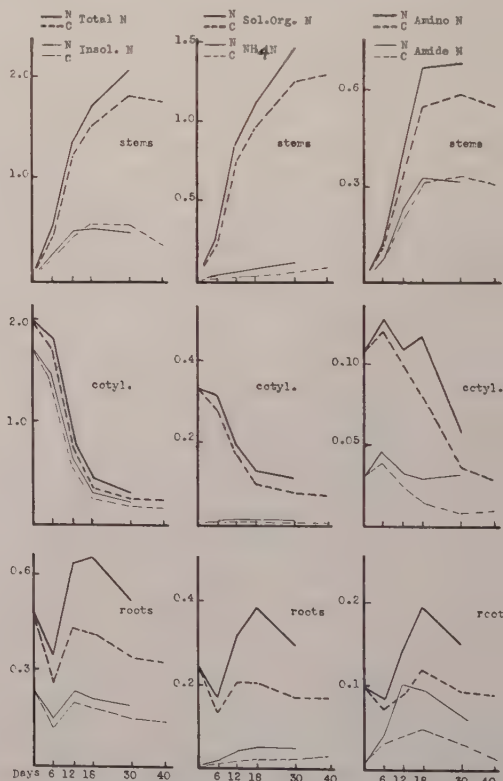


FIG. 4. Peanut. Nitrogen fractions in grams per hundred seedlings.

ent in the hemicelluloses and to a less extent in the starch in the cotyledons and roots. The trends in the nitrogen relations (fig. 4) were not as striking as those noted in the pumpkin. The initial drop in the nitrogen-fraction curves in the roots is due to the fact that the plumules and upper hypocotyls were not separated from the radicles at the initial sampling period. The relative rates of absorption and utilization of ammonium were considerably less than those noted in the pumpkin. Ammonium nutrition resulted in significant increases in protein at 12 days, but not at the expense of the protein reserves, owing to presence of adequate carbohydrate reserves. Marked increases in the soluble organic nitrogen fractions in the roots were favored by ammonium nutrition until the 18-day period. There seems to have been considerable translocation of the organic nitrogen from the roots to the tops in both series during the 18- to 30-day period. The decided increase in soluble organic nitrogen of the stems during this period seems to have been in part due to the movement of organic nitrogen from the cotyledons. The leveling of the organic nitrogen curves during this period suggests that the marked increase in the stems must have been caused by inward movement of organic nitrogen from the roots. Although the synthetic processes were materially inhibited during the carbohydrate starvation period (18 to 30 days) there seems to have been no serious breakdown of total organic nitrogen or of amides by ammonium nutrition even at the point of serious carbohydrate deficiency (fig. 4).

EXPERIMENTS WITH WHITE LUPINE

Seedlings were sampled at 6, 12, and finally at 16 days, when ammonium injury appeared characterized by typical starvation symptoms as previously described for the pumpkin (desiccation of the leaflets). The analytical data are presented graphically in figures 5 and 6. Ammonium nutrition resulted in better growth of roots, and inferior growth of tops, which was true throughout all stages. These relations were also noted in the yellow lupine experiments. Considerable enlargement of the lower hypocotyl resulted from ammonium nutrition. This enlargement was not caused by increased secondary thickening, but by enlargement of pith and cortical cells. Better development of lateral roots was favored by ammonium nutrition. Although significant decreases in sugars were noted in all organs of ammonium-nourished seedlings the hemicelluloses were not significantly less than in the cotyledons of the control except at starvation. The synthesis of organic nitrogen from ammonium proceeded rather smoothly during the early stages in all organs, especially the roots. However, ammonium nutrition resulted in a serious disruption of synthetic processes in the carbohydrate starvation period (12 to 16 days); amides were remarkably decreased in all organs of the plants, and ammonium accumulated in considerable quantities

in the stems and roots; however, the amino nitrogen simultaneously increased in these organs. During this period amides and amino nitrogen increased in the stems and cotyledons but decreased slightly in the roots of the controls.

EXPERIMENTS WITH YELLOW LUPINE

Samplings were made at 3, 6, and finally at 9 days when carbohydrate starvation symptoms were apparent in the ammonium-nourished seedlings. Data obtained are presented graphically in figures 7 and 8. Very narrow differences in fresh weight of the various organs were obtained in the two series during the early stages (3 days). At 6 days the growth of the ammonium epicotyls was noticeably stunted, which is reflected in fresh weight difference in the stems in figure 7. This difference was much more pronounced at carbohydrate starvation (9 days). The depletion of sugars by ammonium nutrition was very marked at 3 days, especially in the roots, in which regeneration of hemicelluloses was materially hindered especially

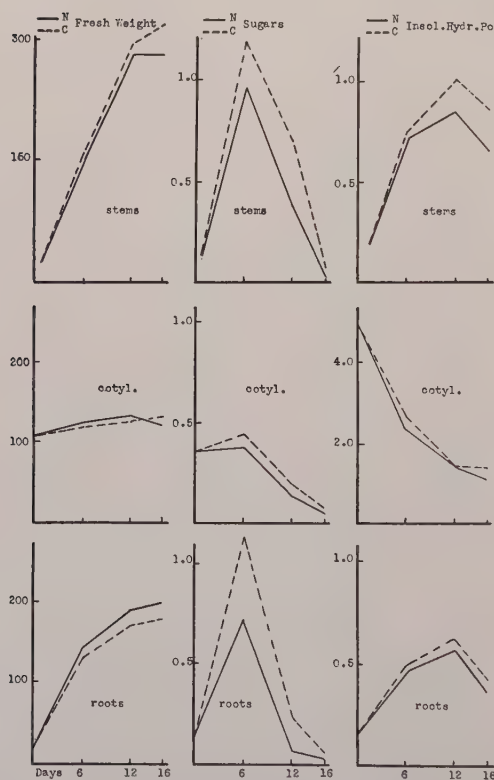


FIG. 5. White lupine. Fresh weights and carbohydrates in grams per hundred seedlings.

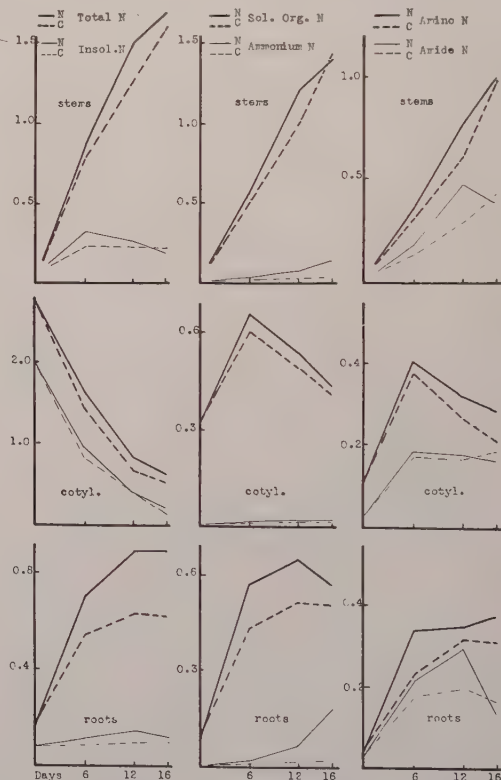


FIG. 6. White lupine. Nitrogen fractions in grams per hundred seedlings.

during later stages. Hemicelluloses seemed to have decreased in the stems during the 3- to 6-day period.

In the early stage, ammonium effected a significant increase in organic nitrogen, especially in the roots, where there was a significant increase in protein nitrogen. By the sixth day this increase in protein nitrogen had disappeared; however, during this same period there was a marked increase in amide and amino nitrogen which were favored by ammonium nutrition. This trend was not as great in the stems during this period. During the period of carbohydrate deficiency (6 to 9 days) the synthetic processes were seriously disrupted, especially in the stems, as a result for the most part of carbohydrate deficiency. Both amide and amino nitrogen decreased, and ammonium accumulated considerably. During this same period there was a remarkable increase in amide and amino nitrogen in the control stems.

These findings concerning the yellow lupine constitute a more complete story of the metabolism of the etiolated yellow lupine seedlings than the reports by PRIANISCHNIKOW and his co-workers (29, 30, 31). These investigators sampled the etiolated yellow lupine seedlings at ten days and did not make samplings at intervals during the life period. As indicated in figures 7 and 8, the life period of the etiolated yellow lupine seedlings supplied with ammonium was 9 days. After having determined the life period of the seedlings in preliminary trials, samplings were made at convenient intervals which were considered of interpretative value in respect to the various internal changes which occur during the progress of growth.

As indicated in the remarks concerning the other species of seedlings employed in these studies, a similar procedure of sampling the material for analysis was carried out. From the foregoing studies of ammonium nutrition, the following trends were noted in the various species of seedlings employed:

(1) In the early stages of growth, when available carbohydrates are abundant, ammonium is readily absorbed and utilized, resulting in protein synthesis which is especially evident in the roots. There is a tendency for growth of the seedlings to be favored by ammonium. Duration of these early stages varies considerably with the various species and seems to be associated somewhat with the type and amount of food reserves stored in the kernel.

(2) In the intermediate stages when available carbohydrates are less plentiful, although ammonium is absorbed and utilized, proteins are broken down and amides accumulate. During this period ammonium has little effect upon growth.

(3) During the carbohydrate starvation period, when available carbohydrates have become seriously depleted, ammonium is neither utilized, nor absorbed; proteins continue to be broken down; amides are decomposed; ammonium accumulates; and there may, or may not be, an increase in am-

monium nitrogen. There is a tendency for leaching of nitrogen from the seedlings to occur during this period as will be shown later. Carbohydrate starvation becomes progressively evident as characterized by cessation of growth followed by desiccation of the aerial organs. The accumulation of ammonium during this period is a clue to the breakdown of organic nitrogen compounds resulting from extreme deficiency of available carbohydrates.

COMPARATIVE STUDY OF SEEDLINGS

The comparative growth responses of the various species of seedlings supplied with ammonium are of interest with respect to their relationship to the food reserves. In figure 9 the fresh weights are presented on a comparable basis as a criterion of growth response.

During the early and intermediate stages of growth of the seedlings when sugars were plentiful (table IV), the ammonium absorption curve (fig. 10) closely paralleled the ammonium utilization curve (fig. 11) for the respective

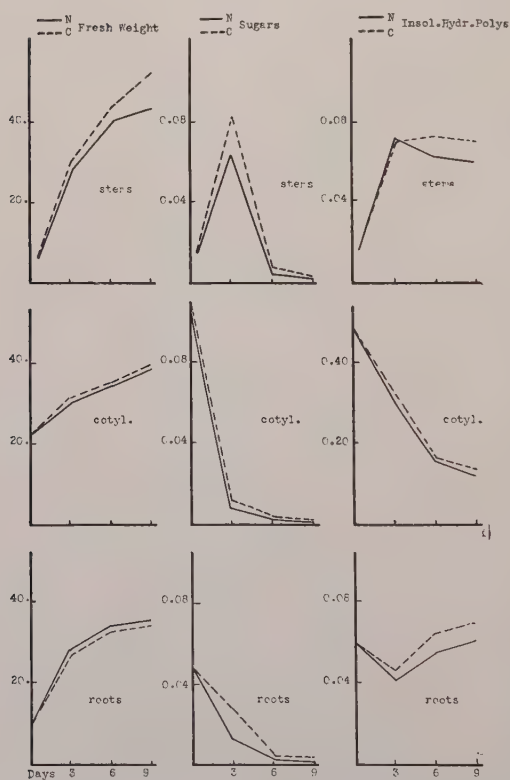


FIG. 7. Yellow lupine. Fresh weights and carbohydrates in grams per hundred seedlings.

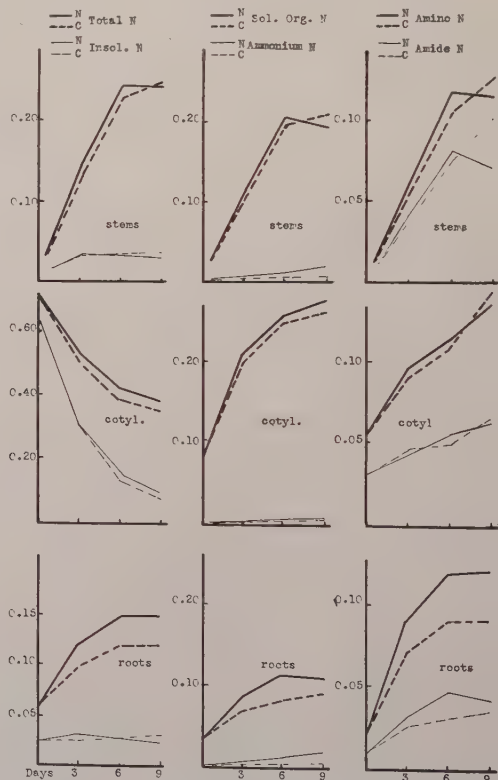


FIG. 8. Yellow lupine. Nitrogen fractions in grams per hundred seedlings.

species. During the later stages of growth as the available carbohydrate supply became exhausted (table IV), these curves diverged from each other due to the cessation of ammonium absorption in conjunction with the continued breakdown of the simple organic nitrogen compounds which results in ammonium accumulation (table III). The leaching of nitrogen from the seedlings during the carbohydrate starvation period is indicated in figure 10.

Although in the early stages of growth the concentrations of sugars in the stems and roots of the peanut seedlings are approximately six times the concentration of sugars in the stems and roots of the pumpkin seedlings (table IV), the rate of ammonium utilization by the peanut seedlings is about one-third the rate of ammonium utilization by the pumpkin seedling. This

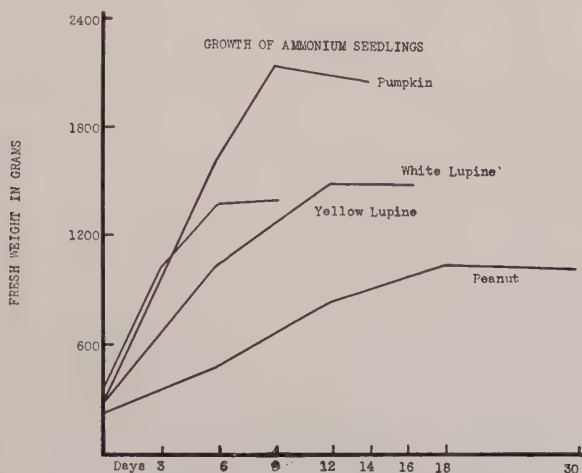


FIG. 9. Fresh weights of seedlings supplied with ammonium nitrogen (calculated on comparable basis as fresh weight in grams produced from a hundred grams of dry ungerminated kernels).

apparent inconsistency seems to be caused by inherent differences in the metabolic characteristics of the species not measured by the present chemical methods. It is difficult to understand why the peanut seedling should be so sluggish in its response to ammonium when the sugar concentration (table IV) in the actively growing seedling is so much greater than in the very responsive pumpkin seedling. The sluggishness of the peanut seedling cannot be attributed to an inferior absorption mechanism, for the concentrations of ammonium in the seedling of the peanut and pumpkin seedlings are approximately the same (table III). The behavior of the peanut seedlings, and their relatively long life period closely resemble the *Phaseolus* seedling (2) which represented the typical starchy legume type.

The apparent sluggishness of the peanut seedlings with respect to ammonium absorption and utilization seems to be associated in part with the

sluggish growth characteristics (fig. 8). On the other hand the remarkable response of the pumpkin seedlings with respect to ammonium absorption and utilization appears to be associated with the relatively rapid growth rate (fig. 8). This rapid growth rate seems to be related to the rapid conversion of the fatty reserves into sugars, which are utilized in growth and respiration but are not stored as starch, as occurs with a considerable portion of the sugars in the early stages of growth of the peanut seedlings.

The relative rates of respiration of the various species of seedlings should have thrown considerable light upon their comparative responses to am-

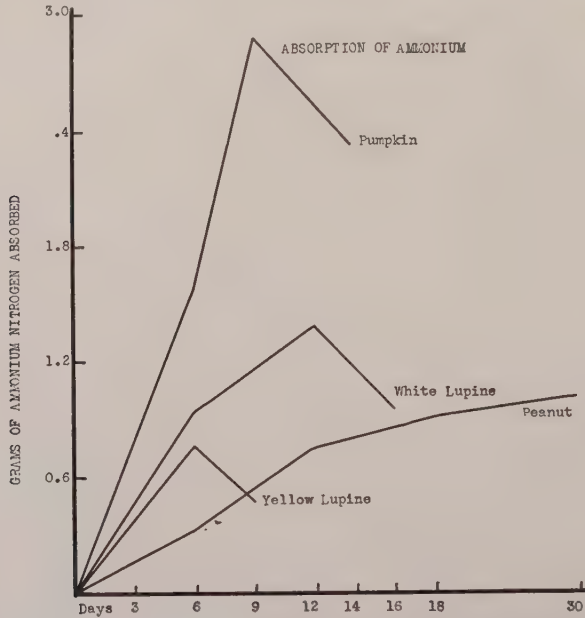


FIG. 10. Absorption of ammonium nitrogen by etiolated seedlings (calculated as difference in total nitrogen between the ammonium seedlings and control seedlings on comparable basis of a hundred grams of dry ungerminated kernels).

monium. Of special interest would be a comparative study of the respiration of the peanut and pumpkin seedlings of which the ungerminated kernels are high in oil. The rapid conversion of the oil to starch makes the peanut a starchy type, whereas there is distinctly no such conversion in the pumpkin seedlings which are so responsive to ammonium. A study of the respiration of the yellow lupine seedlings would be of interest in connection with the high protein reserve in the ungerminated kernels.

A comparative relationship of the soluble and total solids in the seedlings is presented in table V. In order more conveniently to make comparisons, the data pertaining to the solids in the seedlings have been purposely con-

densed to the form in which they appear in this table. Data pertaining to all of the species of seedlings appear for the 6-day period. And finally, data on solids at the period of extreme carbohydrate starvation, of the ammonium-nourished seedlings appear for the respective species. At the 6-day period the pumpkin seedlings increased in total solids, but markedly decreased thereafter. There seems to be no consistent trend with respect to the effect of ammonium upon the total solids. In the case of the pumpkin, however, ammonium resulted in a very significant decrease in total solids at the 14-day period. This seems to be the resultant of increased respiration because of the favorable effect of the ammonium upon the growth of these seedlings. Of

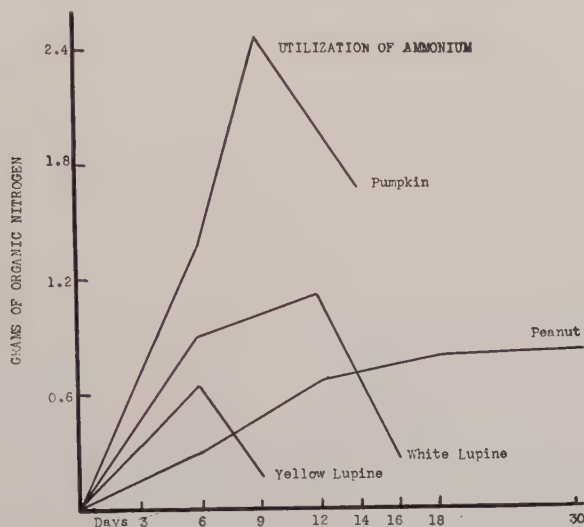


FIG. 11. Utilization of ammonium nitrogen by etiolated seedlings (calculated as difference in organic nitrogen between the ammonium seedlings and control seedlings on comparable basis of a hundred grams of dry ungerminated kernels).

the respective species, the loss of total solids during the life period was greatest in the case of the peanut, which seems to be associated with its long life period and the nature of the food reserves in its kernels.

General discussion

A few remarks in regard to the present status of the nitrogen relationships in seedlings are now presented for the purpose of orientation. One of the most striking observations has been the accumulation of amides in shoots of growing seedlings (especially of the Leguminosae), when germinated in darkness for two or three weeks. This observation has been the subject of much discussion in recent years. In some seedlings, the proportion of amides to amino acids is fairly uniform at certain stages of germination, while at other

TABLE V

SOLIDS IN SEEDLINGS AS PERCENTAGE OF DRY UNGERMINATED KERNELS (N = AMMONIUM-
NOURISHED SEEDLINGS; C = CONTROL SEEDLINGS)

PERIOD OF GROWTH	PUMPKIN		PEANUT		WHITE LUPINE		YELLOW LUPINE	
	SOLUBLE SOLIDS	TOTAL SOLIDS	SOLUBLE SOLIDS	TOTAL SOLIDS	SOLUBLE SOLIDS	TOTAL SOLIDS	SOLUBLE SOLIDS	TOTAL SOLIDS
	%	%	%	%	%	%	%	%
6 days N	46.7	104.6	22.6	96.4	45.9	95.9	47.8	80.8
C	44.7	101.6	22.8	96.6	45.4	93.0	49.4	82.8
9 days N	—	—	—	—	—	—	49.5	79.5
C	—	—	—	—	—	—	49.4	79.5
14 days N	36.2	81.3	—	—	—	—	—	—
C	32.9	87.8	—	—	—	—	—	—
16 days N	—	—	—	—	40.1	73.5	—	—
C	—	—	—	—	36.9	72.7	—	—
18 days N	—	—	33.6	77.8	—	—	—	—
C	—	—	32.2	77.9	—	—	—	—
30 days N	—	—	24.6	67.8	—	—	—	—
C	—	—	24.4	68.8	—	—	—	—

stages amides prevail. Recently PUCHER (28) and VICKERY (62) have demonstrated the widespread occurrence of glutamine in seedlings. Seedlings of legumes are rich in asparagine, while high oil-containing seeds, such as the sunflower, pumpkin, and castor bean, produce considerable amounts of glutamine. It has been shown that the amides accumulate in greater amounts than can be accounted for by direct hydrolysis of reserve proteins. Recently KLEIN and TAUBÖCK (12, 13) have emphasized the importance of basic amino acids which accumulate in considerable amounts in certain types of seeds on germination, with special attention given to arginine. Little is known concerning the interrelationships and significance of these facts.

SCHULZE (47-53) and PRIANISCHNIKOW (29-34, 39) have independently conducted a long series of investigations in which attempts were made to throw light on these phenomena. Both agree that much asparagine is formed during germination, and that under favorable conditions it is used in protein synthesis. Ammonium is formed by deaminization, probably by oxidation of amino acids. The origin of succinic acid, which they postulate plays an important rôle in asparagine formation, is somewhat obscure. SCHULZE (50) is inclined to believe that amino acids $\rightarrow$ ammonia breakdown with subsequent formation of asparagine is to a large extent an essential process, and that asparagine is the chief source of nitrogen for protein synthesis.

PRIANISCHNIKOW (29-37, 39) regards asparagine chiefly as a by-product in which form the surplus, and harmful though valuable ammonium may be stored for future use. He believes that the accumulation of amides is dependent upon a proper carbohydrate-nitrogen balance. When excess carbohydrates are present, as in photosynthesis, asparagine disappears owing to favorable conditions for protein synthesis. When this condition exists, asparagine serves as a translocatory form of ammonium and is utilized in protein synthesis. CHIBNALL is in favor of this view (3, 4). When a reasonable amount of carbohydrates is present, as in the early growth stages of etiolated seedlings, there is an accumulation of amides, for conditions are not favorable for maximum protein synthesis. But when extreme deficiency of carbohydrates is obtained, there is no opportunity for the accumulation of asparagine, owing to lack of oxidation products upon which asparagine synthesis depends. Under these latter conditions, ammonium accumulates, thereby becoming toxic. The writer seriously questions the validity of such an inference regarding the toxicity of ammonium, because it may accumulate considerably in plants without injury (14, 44). He prefers to think of the pathological condition during which ammonium accumulates as caused by an unfavorable complex of conditions in which depletion of available carbohydrates plays a predominant rôle, and that the accumulation of ammonium is probably a resulting phenomenon, but not the cause of the condition.

MURNEEK (20) in recently reviewing the rôle of asparagine and related substances in plants has not seemed to have critically evaluated the findings and conclusions of PRIANISCHNIKOW. It seems that, although amides accumulate under certain conditions, there is little justification for assuming that amides serve as efficient detoxicants of ammonium. It is believed that further studies of the so-called "acid plants" (14, 43, 44) subjected to ammonium nutrition should stimulate new lines of thought in regard to these phases of nitrogen metabolism. The writer prefers to consider the accumulation of asparagine to be a resultant of certain carbohydrate-nitrogen relations and that it really does not serve a purpose in the detoxification of ammonium.

Owing to the lack of a definite understanding of nitrogen relations existing in normal seedlings, it becomes very difficult to make a satisfactory analysis of the results obtained in this investigation. Accordingly, some interpretations and generalizations in regard to the conditions brought about by ammonium nutrition in the etiolated seedlings are of necessity partially speculative. In order to grow plants under controlled conditions, they must necessarily be placed under somewhat artificial conditions. Even though the seedlings employed in this study were grown under extremely artificial conditions (40), it is believed that a proper analysis of the controlled environmental factors in conjunction with a careful consideration of the differences brought about by the variable (ammonium supply), should throw light upon

certain phases of plant metabolism which may not otherwise be obtained. From this viewpoint, this study was deemed justifiable in the light of the present confusion regarding the rôle of certain nitrogen compounds in the normal metabolism of plants.

In these experiments it was found that growth response of the various seedlings supplied with ammonium salts was in part dependent upon the nature of the food reserves. In this connection, REID (41) has mentioned some of the practical implications arising from studies of the response of etiolated seedlings to nitrogen supplied in the form of nitrates. Owing to the wide differences in the morphological characteristics, as well as to the complex hereditary tendencies inherent in the various species, it becomes somewhat far-fetched or questionable definitely to assign the different types of response (growth and certain internal conditions measurable by present chemical methods) to the type and amount of food reserves existing in the kernel. Certainly the hereditary tendencies in conjunction with modified physico-chemical relationships must play an important part in determining the responses of various seedlings to ammonium nutrition. The trends noted in these species would in no wise be expected to occur in the "acid plants" of RUHLAND and WETZEL (43, 44), especially with respect to the amide relations.

A further complication in making certain generalizations in such an investigation is the lack of knowledge as to the exact nature of the food reserves in the seeds; for example, ether extract and hemicelluloses are empirical terms assigned to groups of substances (more or less heterogeneous) about which little is specifically known. MILLER (19) gives a comprehensive historical review of the earlier investigations pertaining to the ether extract as storage material in the seeds and the changes it undergoes during germination. In this connection he (19) has studied quite extensively the changes occurring during the germination of sunflower seedlings. Little work has been done on hemicelluloses of seeds and seedlings, aside from the studies carried out by SCHULZE (1889-1910). Reserve protein is an ambiguous term, in that it is so closely interrelated to non-reserve protein that a differentiation of the two forms as they occur in the storage organs is beyond the scope of present methods of chemical analysis (22). Furthermore, little is known concerning the nature and rôle of non-protein nitrogen in the seed and seedlings, especially the "rest nitrogen."

The question as to the mechanism of ammonium absorption by plants has received but little consideration. NEVIUS (17, 18) believes that ammonium absorption increases in neutral or slightly alkaline solutions, owing to the greater degree of hydrolytic cleavage of the salts and correspondingly greater ammonia tension, which determines to a large extent the physiological effects of ammonium salts. Furthermore, he states that the injury may result from

a too rapid accumulation of ammonium under neutral or slightly alkaline conditions. NAFTEL (21) believes that ammonium alters the colloidal complex of the protoplasm which in turn affects the rate of absorption of ammonium. The study of interionic absorption, to say the least, involves many complications (PIRSCHLE, 26, 27 and NEVIUS, 17, 18). Although in accordance with absorption principles, these explanations are quite theoretical and the assumptions call for experimental verification. HOAGLAND (8), LUNDEGÅRDH (16), and STEWARD (56) have briefly reviewed some recent papers pertaining to ionic absorption. HOAGLAND and BROYER (9) believe that the absorption of ions is dependent upon metabolic activity. This view seems highly speculative. HOAGLAND (8) also emphasizes the importance of the buffering system of plant sap as a factor in ionic absorption. PRIANISCHNIKOW (36, 37) has recently shown that the rates of absorption of ammonium and nitrate by etiolated seedlings from single salt solutions of ammonium nitrate varies considerably with the pH of the culture medium and the age of the seedlings. However, no attempt was made to determine the extent of utilization. The author questions the value of such experiments in which single salt solutions are employed.

One thinks of the ammonium ion, after having entered the root of seedlings, as affecting directly or indirectly innumerable processes which in turn have a direct bearing on metabolism and growth. Various physico-chemical relationships in the cellular structure must be altered by the ammonium ion, such as permeability, translocation, pH, and interionic relations within the plant tissue. HOLLEY *et al.* (10) have shown that ammonium definitely reduced calcium absorption, which in turn may affect materially the mobilization of carbohydrates and other substances. The physico-chemical effects of ammonium may be expected to modify many metabolic processes such as hydrolytic reactions (hydrolysis of reserves, etc.), translocation of hydrolytic products, mobility and lability of sugars, amino acids, etc., respiration (in this investigation certain relations concerning dry weights seemed to suggest that respiration was little affected by ammonium nutrition under the condition of these experiments), and synthetic processes, which in turn largely determine the organo-chemical response of seedlings to ammonium nutrition.

In this nitrogen investigation of etiolated seedlings supplied with ammonium, injury resulted from the extreme deficiency of available carbohydrates. The concentration of ammonium did not increase greatly when carbohydrate deficiency became very severe; however, during this period leaching of nitrogen occurred from all species studied except perhaps the peanut seedlings. KULTZSCHER (14) concludes that the reaction of the cell sap is important in the storage of ammonium. More information is seriously needed concerning the extent and conditions under which ammonium may accumulate in plants without injury in order to more satisfactorily evaluate

the rôle of ammonium in plants. More extensive organo-chemical investigation supplemented with histological and physico-chemical studies are needed in order to obtain a more satisfactory conception of the conditions associated with so-called "ammonium injury" which in a large measure seems to be caused by the depletion of available carbohydrates.

Summary and Conclusions

1. A brief resumé of PRIANISCHNIKOW's investigations pertaining to the rôle of ammonium in the nitrogen metabolism of etiolated seedlings and a critical analysis of his methods are included in the introduction.

2. With the adaptation of improved methods this investigation involves a further study of the effects of ammonium nutrition on the carbohydrate and nitrogen relations of etiolated seedlings. Seedlings of the following species were employed:

Cucurbita pepo (high oil reserve) pumpkin

Arachis hypogaea (high oil reserve) peanut

Lupinus albus (high hemicellulose and protein reserve) white lupine

Lupinus luteus (high protein reserve) yellow lupine

The seedlings were fractionated into stems, cotyledons, and roots for the analyses which were made at suitable intervals until the plants showed carbohydrate starvation symptoms.

3. In this investigation the conditions which resulted during ammonium nutrition cannot be ascribed to physiological acidity. In the light of modern principles of plant nutrition the methods employed by PRIANISCHNIKOW render the basis for his classification of seedlings unjustifiable.

4. The rate of absorption and utilization of ammonium was most rapid in the case of pumpkin. Although the oily reserve was rapidly converted into sugars no starch was formed therefrom. There was very little reversion of the sugars to fat in the seedlings. A marked depletion of sugars was associated with the rapid utilization of ammonium. Growth was favored by this association in the earlier stages during which time protein formation or regeneration was favored by ammonium nutrition. At the stage of extreme carbohydrate starvation (fourteen days) the protein and amide fractions were materially diminished in the stems while ammonium accumulated.

5. Although the food reserves of the peanut kernel are somewhat similar to those of the pumpkin, the response of the peanut seedlings to ammonium was very sluggish. During the early stages of germination and growth of the seedlings the oily reserve was rapidly converted into sugars, and in turn much of the sugar was converted into starch in the cotyledons and in the primary roots. At the time of carbohydrate starvation starch had entirely disappeared from the primary roots; however, a small amount of starch remained in the cotyledons. Even though the peanut kernels were high in oil

reserve the peanut seedlings behaved as physiological starchy legumes. The accumulation of amides favored by ammonium nutrition in the roots during the early stages was associated with a decrease in the sugars. Amides later decreased as sugars were further depleted. The ammonium peanut seedlings showed carbohydrate starvation symptoms at thirty days, while the controls showed similar symptoms at forty days.

6. Ammonium was utilized especially by the roots until the twelfth day in the case of the white lupine. Ammonium nutrition resulted in a marked depletion of amides in all organs during the carbohydrate starvation period (12 to 16 days) due to the exhaustion of the sugar supply resulting from earlier utilization of ammonium. Ammonium rapidly increased during the carbohydrate starvation period.

7. The yellow lupine seedlings utilized ammonium in the early stages (3 and 6 days). During the carbohydrate starvation period (6 to 9 days) ammonium was neither utilized nor absorbed. The amides were broken down and ammonium accumulated as a result of extreme depletion of available carbohydrates.

8. All the seedlings studied absorbed and utilized ammonium during early stages of germination while the synthetic processes were disrupted during the carbohydrate starvation periods when ammonium accumulated as a result of the breakdown of organic nitrogen because of the exhaustion of available carbohydrates. Ammonium injury appeared to be associated with carbohydrate depletion but is not ascribed to any specific condition.

9. In this investigation it appears to have been demonstrated that there are at least three distinct internal conditions with intergradations which are associated with the response of the etiolated seedlings to ammonium nutrition, namely:

(1) In the early stages of growth when available carbohydrates are abundant ammonium is readily absorbed and utilized resulting in protein synthesis which is especially evident in the roots. There is a tendency for growth of the seedlings to be favored by ammonium. The degree of response and duration of these early stages varies considerably with the various species and seems to be associated somewhat with the type and amount of food reserves stored in the kernel.

(2) In the intermediate stages when available carbohydrates are less plentiful, though ammonium is absorbed and utilized, proteins are broken down and amides accumulate. During this period ammonium has little effect upon growth.

(3) During the carbohydrate starvation period, when available carbohydrates have become seriously depleted, ammonium is neither utilized, nor absorbed; proteins continue to be broken down; amides are decomposed; ammonium accumulates, and there may, or may not be an increase in amino

nitrogen. There is a tendency for leaching of nitrogen from the seedlings to occur during this period. Carbohydrate starvation becomes progressively evident as characterized by cessation of growth followed by desiccation of the aerial organs. The accumulation of ammonium during this period is due to the breakdown of organic nitrogen compounds resulting from extreme deficiency of available carbohydrates.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Methods of analysis. 3rd ed. Washington. 1930.
2. BURKHART, L. Metabolism of etiolated seedlings as affected by ammonium nutrition. *Plant Physiol.* **9**: 351-358. 1934.
3. CHIBNALL, A. C. Investigations in the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. *Biochem. Jour.* **16**: 344-362. 1922.
4. ————. *Ibid.* VI. The rôle of asparagine in the metabolism of the mature plant. *Biochem. Jour.* **18**: 395-404. 1924.
5. DAVIDSON, A. W., and SHIVE, J. W. Determination of the nitrogenous fractions in vegetative tissues of the peach. *Plant Physiol.* **10**: 71-92. 1935.
6. DENNY, F. E. Eliminating the use of calcium carbonate in preparing plant tissue for analysis. *Contrib. Boyce Thompson Inst.* **5**: 103-114. 1933.
7. ————. Improvements in methods of determining starch in plant tissue. *Contrib. Boyce Thompson Inst.* **6**: 129-146. 1934.
8. HOAGLAND, D. R. Mineral nutrition of plants. *Annual Rev. Biochem.* **2**: 471-484. 1933.
9. ————, and BROYER, T. C. Metabolic activities and accumulation of mineral nutrients by barley root cells. Abstract of paper presented before the American Society of Plant Physiologists, December, 1932.
10. HOLLEY, K. T., PICKETT, T. A., and DULIN, T. G. A study of ammonia and nitrate nitrogen for cotton. I. Influence on absorption of other elements. *Georgia Agr. Exp. Sta. Bull.* 169. 1931.
11. HULME, A. C. Biochemical studies in the nitrogen metabolism of the apple fruit. I. The estimation of amino-nitrogen by the Van Slyke method in the presence of tannin. *Biochem. Jour.* **29**: 263-271. 1935.
12. KLEIN, G., and TAUBÖCK, K. Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. *Biochem. Zeitschr.* **251**: 10-50. 1932.
13. ————, and ————. Argininstoffwechsel und Harnstoff-

- genese bei höheren Pflanzen II. *Biochem. Zeitschr.* **255**: 278–286. 1932.
14. KULTZSCHER, M. Die biologische NH_3 -Entgiftung in höheren Pflanzen in ihrer Abhängigkeit von der Wasserstoffionenkonzentration des Zellsaftes. *Planta* **17**: 699–757. 1932.
 15. LIVINGSTON, B. E. Personal communication.
 16. LUNDEGÅRDH, H. Mineral nutrition of plants. *Annual Rev. Biochem.* **3**: 485–500. 1934.
 17. MEVIUS, W. Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentrationen. *Planta* **6**: 379–455. 1928.
 18. ———, and ENGEL, H. Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentrationen. II. *Planta* **9**: 1–83. 1929.
 19. MILLER, E. C. A physiological study of the germination of *Helianthus annuus*. *Ann. Bot.* **26**: 889–901. 1912.
 20. MURNEEK, A. E. Physiological rôle of asparagine and related substances in metabolism of plants. *Plant Physiol.* **10**: 447–464. 1935.
 21. NAFTEL, J. A. The absorption of ammonium and nitrate nitrogen by various plants at different stages of growth. *Jour. Amer. Soc. Agron.* **23**: 142–158. 1931.
 22. PAULI, W. The chemistry of the amino acids and the proteins. *Annual Rev. Biochem.* **3**: 111–132. 1934.
 23. PHILLIPS, T. G. The determination of sugars in plant extracts. *Jour. Biol. Chem.* **95**: 735–742. 1932.
 24. ———, SMITH, T. O., and DEARBORN, R. B. The effect of potassium deficiency on the composition of the tomato plant. *New Hampshire Agr. Exp. Sta. Tech. Bull.* 59. 1934.
 25. ———, *et al.* The determination of nitrogen in relatively simple compounds. *Plant Physiol.* **2**: 205–211. 1927.
 26. PIRSCHLE, K. Nitrate und ammonium Salze als Stickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. III. *Planta* **14**: 583–676. 1931.
 27. ———, and MENGDEHL, H. Ionenaufnahme aus Salzlösungen durch die höhere Pflanze. *Jahrb. wiss. Bot.* **74**: 297–363. 1931.
 28. PUCHER, G. W., VICKERY, H. B., and LEAVENWORTH, C. S. Determination of ammonia and of amide nitrogen in plant tissue. *Ind. & Eng. Chem. Anal. Ed.* **7**: 152–156. 1935.
 29. PRIANISCHNIKOW, D. The rôle of ammonia in the metabolism of nitrogenous substances in plants. *Internatl. Rev. Sci. and Pract. Agr.* **8**: 204–216. 1917.
 30. ———. Das Ammoniak als Anfangs- und Endprodukt des Stickstoffumsatzes in den Pflanzen. *Landw. Vers.-Sta.* **99**: 267–286. 1922.

31. ————. Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. d. bot. Ges. **40**: 242–248. 1922.
32. ————. Zur Frage über die Bedeutung des Calciums für die Pflanzen. Ber. d. bot. Ges. **41**: 138–144. 1923.
33. ————. Asparagin und Harnstoff. Biochem. Zeitschr. **150**: 407–423. 1924.
34. ————. Sur le rôle de l'asparagine dans les transformations des matières azotées chez les plantes. Rév. Gén. Bot. **36**: 108–122. 1924.
35. ————. Zur Frage nach der Ammoniakernährung von höheren Pflanzen. Biochem. Zeitschr. **207**: 341–344. 1929.
36. ————. Über die äusseren und inneren Bedingungen der Ausnutzung des Ammoniakstickstoffs durch die Pflanzen. I. Zeitschr. Pflanzenernähr. Düngung & Bodenk. A **30**: 38–82. 1933.
37. ————. Über die äusseren und inneren Bedingungen der Ausnutzung des Ammoniakstickstoffs durch die Pflanzen. II. Zeitschr. Pflanzenernähr. Düngung & Bodenk. A **33**: 134–169. 1934.
38. ———— and DOMONTOVITCH, M. K. The problem of a proper nutrient medium. Soil Sci. **21**: 327–348. 1926.
39. ———— und SCHULOW, J. Über die synthetische Asparaginbildung in dem Pflanzen. Ber. d. bot. Ges. **28**: 253–264. 1910.
40. PRIESTLY, J. B. Light and growth. II. On the anatomy of etiolated plants. New Phytol. **25**: 145–170. 1926.
41. REID, MARY E. Growth of seedlings in light and in relation to available nitrogen and carbon. Bot. Gaz. **87**: 81–117. 1929.
42. RICHARDSON, G. N. Critique on the biological estimation of amino nitrogen. Proc. Roy. Soc. Lond. B. **115**: 142–169. 1934.
43. RUHLAND, W., and WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. I. *Begonia semperflorens*. Planta **1**: 558–564. 1926.
44. ————, und ————. Zur Physiologie der organischen Säuren in grünen Pflanzen. III. *Rheum hybridum* Hort. Planta **3**: 765–769. 1927.
45. ————, and WOLF, J. Metabolism of carbohydrates and organic acids in plants. (Exclusive of bacteria and fungi.) Annual Rev. Biochem. **3**: 501–518. 1934.
46. SCHLENKER, F. S. Comparison of existing methods for the determination of ammonia nitrogen and their adaptability to plant juice. Plant Physiol. **7**: 685–695. 1932.
47. SCHULZE, E. Ueber Zersetzung und Neubildung von Eiweissstoffen in Lupinenkeimlingen. Landw. Jahrb. **7**: 411–444. 1878.
48. ————. Ueber die Bildungsweise des Asparagins und über die

- Beziehungen der stickstofffreien Stoffe zum Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. **17**: 683-711. 1888.
49. ———. Über die Verbreitung des Glutamins in den Pflanzen. Landw. Vers.-Sta. **48**: 33-55. 1897.
 50. ———. Über den Eiweissumsatz und die Bildungsweise des Asparagins und des Glutamins in den Pflanzen. Zeitschr. physiol. Chem. **26**: 411-426. 1898-99.
 51. ———. Über den Abbau und den Aufbau organischen Stickstoffverbindungen in den Pflanzen. Landw. Jahrb. **35**: 621-666. 1906.
 52. ———, and CASTORO, N. Beiträge zur Kenntnis der Zusammensetzung und des Stoffwechsels der Keimpflanzen. Zeitschr. physiol. Chem. **38**: 199-258. 1903.
 53. ———, and WINTERSTEIN, E. Ueber aus den Keimpflanzen von *Vicia sativa* und *Lupinus albus* darstellbaren Monaminesäuren. Zeitschr. physiol. Chem. **45**: 38-60. 1905.
 54. SMIRNOW, A. I. Über die Synthese der Säureamide in den Pflanzen bei Ernährung mit Ammoniaksalzen. Biochem. Zeitschr. **137**: 1-34. 1923.
 55. SHIVE, J. W., and STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**: 317-323. 1927.
 56. STEWARD, F. C. Mineral nutrition of plants. Annual Rev. Biochem. **4**: 519-544. 1935.
 57. STUART, N. The determination of amino nitrogen in plant extracts. Plant Physiol. **10**: 135-148. 1935.
 58. TIEDJENS, V. A. Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in tomato and apple. Plant Physiol. **9**: 31-57. 1934.
 59. TOTTINGHAM, W. E., *et al.* Chemical analysis of plant tissue. Plant Physiol. **10**: 383-399. 1935.
 60. TRUE, R. H. The function of calcium in the nutrition of seedlings. Jour. Amer. Soc. Agron. **13**: 91-107. 1921.
 61. VICKERY, H. B. The biochemistry of the nitrogenous constituents of the green plants. Annual Rev. Biochem. **3**: 475-484. 1934.
 62. ———, PUCHER, G. W., and CLARK, H. E. Glutamine in the tomato plant. Science n.s. **80**: 459-461. 1934.
 63. WEBSTER, J. E. Changes occurring in stored alcoholic plant extracts. Science n.s. **73**: 77-78. 1931.
 64. ———. Nitrogen changes in stored alcoholic extracts of plant tissues. Plant Physiol. **8**: 166-168. 1933.

